Post-emergent Herbicidal Activity of Cineole Derivatives

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Abstract

Essential oils are being investigated as potential herbicides or to provide leads to new environmentally and socially acceptable herbicides. Novel hydroxy and ester derivatives of 1,8-cineole and 1,4-cineole were synthesised, by chemical and biological methods, and have shown pre-emergence herbicidal activity against annual ryegrass and radish. Effects on post-emergence activity of these derivatives, as well as 1,8-cineole, eucalyptus oil and the carboxylic acids from which the esters were derived, against annual ryegrass and radish, are reported here. Results suggest that reduced root and shoot growth observed in pre-emergence herbicidal bioassays were due to post-emergence activity rather than delayed germination. All tested substances had a dose-dependent, post-emergence herbicidal activity against annual ryegrass and radish with many derivatives showing improved activity relative to 1,8-cineole and high-cineole eucalyptus oil. However, results do not support the postulate that cineole esters would be more active than their respective carboxylic acid and the hydroxy

cineole. Phytotoxicity of ester derivatives may be due to metabolic cleavage of the esters to the hydroxy cineole and carboxylic acid within the plant.

Keywords: Eucalyptus oil, cineole, hydroxy cineole, monoterpenes, phytotoxicity.

Introduction

The decrease in crop yields in agricultural systems and reduction in biodiversity in natural areas of vegetation due to weeds requires a range of strategies to reduce their impacts. Prior to the mid-twentieth century, strategies to manage weeds were primarily non-chemical, categorised as either mechanical or cultural (Kohli et al. 2006). Mechanical methods such as harrowing and inter-row hoeing together with cultural methods like fertilizer placement, seed vigour, seeding rate and competitive varieties provide encouraging results but long-term solutions require consideration at the cropping system level. In low-external input or ecologically sustainable farming practices weed control may be most effectively approached by examining interactions among system components and agricultural practices occurring in the current crop, the subsequent crop and between crops (Bàrberi 2002; Melander et al. 2005). For example, the slower release of nutrients from organic fertilisers compared to synthetic fertilisers delays weed emergence in turn leading to competition between the crop and weeds occurring later and with the potential for effects to carry over to the next growing season and likely causing changes in the weed community (Bastiaans and Drenth 1999; Liebman 2000; McCloskey et al. 1996). These approaches are ecologically sustainable but can be labour intensive and may not provide the level of weed control needed to give sufficient crop yields to feed the world's population (Gianessi 2009). As well, as understanding of ecosystems has developed, biological control agents have been used but they are usually targeted at a particular species, they can be slow to give an acceptable level of control and usually they are not applicable to cropping systems.

Thus for cropping systems it may be necessary to use chemical weed control to reduce the damage to an acceptable level but herbicide applications have

drawbacks including loss of non-target organisms from microbes to vertebrates due to toxic soil and water residues which can cause indirect ecological problems (Lewis et al. 2009; Wang et al. 2010; Willemsen and Hailey 2001), possible impacts on human health from residues in foods exposed to these chemicals (Chade et al. 2006; Fantke et al. 2012; Rouimi et al. 2012) and development of herbicide resistance in weed species (Heap 2007).

Plant-sourced compounds that exhibit phytotoxicity, and so may act as herbicides or provide leads to novel herbicides, have the potential to avoid some of these problems. Their half-lives are likely to be relatively short thus reducing problems of residues in food and water, and they may have novel modes of action thus potentially addressing problems of resistance to current herbicides (Duke et al. 2002).

Essential oils and their constituents, including monoterpenes, are plant secondary metabolites being increasingly studied for their allelopathic, herbicidal, insecticidal, acaracidal and other biological activities. The varied ecological roles of monoterpenes include reduced susceptibility to insect herbivory, attractants for pollinators (Ibanez et al. 2010) and suppression of germination of competing plants. There are many reports of the insecticidal activity of essential oils and their monoterpene constituents, including 1,8-cineole. Edwards et al. (1993) and Matsuki et al. (2011) observed that increased 1,8-cineole content in leaves reduced herbivory by Christmas beetles. 1,8-Cineole was found to be highly toxic to the grain beetles *Prostephanus truncates*, *Sitophilus granarius*, *S. zeamais* and *Tribolium castaneum* with 100% mortality at 0.5 µL 1,8-cineole per kilogram of grain after 24 hours (Obeng-Ofori et al. 1997). Polatoğlu (2013) also reported toxicity of the essentials oils of *Achillea* species, which have high 1,8-cineole content, to *S. granarius*. 1,8-

Cineole and other monoterpenes have shown toxicity and feeding and oviposition deterrence to the moth species *Helicoverpa armigera* (Hübner), *Spodoptera litura* (F.) and *Chilo partellus* Swinhoe (Koul et al. 2013). The fumigant activity of eucalyptus oils from a number of *Eucalypt* species on adult *Aedes aegypti*, the yellow fever mosquito, correlates with 1,8-cineole content of the oils (Lucia et al 2009) and Lampman et al. (2000) showed mosquito larvicidal activity for 1,8-cineole. Acaricidal activity of essential oils containing 1,8-cineole has been demonstrated with *Rosmarinus officinalis* L., *Salvia officinalis* and Myrtaceae essential oils active against the two-spotted mite *Tetranychus urticae* (Laborda et al. 2013; Miresmailli et al. 2006; Roh et al. 2013). As well, a terpene-based solution containing 1,8-cineole gave a mortality of 96.7% against the western honey bee parasitic mite *Varroa jacobsonii* (Calderone and Spivak 1995).

A wide range of classes of volatile monoterpenes inhibit plant growth (Amri et al. 2012; Apsland 1968; Chaimovitsh et al. 2011; Muller and Muller 1964; Vaughn and Spencer 1993). For example, *Artemisia frigida* has been shown to have inhibiting effects on plant communities in the steppe of northern China with its leaf volatile components comprising mainly monoterpenes, including 1,8-cienole (Li et al. 2011). The high-1,8-cineole essential oil of *Eucalyptus erythrocorys* has demonstrated herbicidal activity against *Sinapis arvensis* L. and *Phalaris canariensis* L. preventing seed germination at oil concentration of 1.5 μL mL^{-1} and delaying and decreasing germination at lower concentrations (Ben Ghnaya et al. 2013).

In the field, the volatility of monoterpenes may limit plant uptake and therefore their effectiveness. There are reports on attempts to address volatility through microencapsulation or nano-formulation of essential oils for the purposes of both herbicidal and insecticidal activity. Nanoparticles loaded with garlic essential oil

and *Mentha* oil nanoparticles have been shown to maintain activity against insects over an extended period (Yang et al. 2009; Kumar et al. 2013) and polyurea microcapsules containing essential oils reduced seed germination relative to controls although were not as effective as neat oils (Scarfato et al. 2007). However, there are few reports of attempts to reduce volatility whilst maintaining herbicidal activity by the synthesis of derivatives of monoterpenes. The derivatives have increased molecular mass to lower vapour pressure and give a slower evaporation rate compared to the parent monoterpene. Vaughn and Spencer (1996) prepared benzyl ether derivatives of a number of monoterpenes for subsequent herbicidal testing but the only report of the synthesis of ester derivatives of monoterpenes such as 1,8-cineole for this purpose is for pre-emergence testing (Barton et al. 2010).

Whilst it is usually preferable to apply herbicides before crops emerge, weeds can emerge to compete with crops that are slow to germinate such as chickpeas or crops may be poor competitors, and so require weed control. Zero or reduced tillage in broad-acre farming has led to volunteer cereals growing with crops and so causing reduced yields (Friesen et al. 1990; O'Donovan 1992; Wilson et al. 2010). Weed control is necessary after crop germination where there are weed species that have lengthy germination periods, grow strongly in autumn and spring, produce large quantities of seed, or have a long lasting seed bank.

An aim of this work was to assess and compare post-emergent herbicidal activity of eucalyptus oil, 1,8-cineole, the major component in the leaf oil of many eucalypts, and hydroxy and ester derivatives of 1,8-cineole and 1,4-cineole. The study investigated whether observed reductions in root and shoot growth of annual ryegrass (*Lolium rigidum*) and radish (*Raphanus sativus* var. Long Scarlet) when seeds were treated pre-emergence with these substances (Barton et al. 2010) was due

to delayed germination or reductions in growth. It was also postulated that on uptake by plants the cineole esters may undergo metabolic cleavage to give the hydroxyl-cineole and the carboxylic acid and so any herbicidal activity of the esters may, in fact, be due to the hydroxyl-cineole and its carboxylic acid. Thus the post-emergence herbicidal activity of the carboxylic acids corresponding to the esters was also assessed. For the purposes of the work reported here, pre-emergence activity was defined as herbicidal activity preventing seed germination and post-emergent activity was defined as activity preventing or reducing further growth after emergence of the radicle and plumule.

Materials and Methods

Chemicals. All chemicals were purchased from standard commercial suppliers. Eucalyptus oil (96% v/v 1,8-cineole) was obtained from Kalannie Distillers, Kalannie Western Australia. The oil was from *Eucalyptus kochii* subsp *horistes* and *Eucalyptus kochii* subsp. *Plenissima*.

Synthesis of 1,8-Cineole and 1,4-Cineole Derivatives. Cineole derivatives were prepared as described in Barton et al. (2010) and references therein.

Seed Sources. Annual ryegrass seeds (*Lolium rigidum*) were obtained from the Wongan Hills Research Station 2EA, Western Australia, in November 2002 and radish seeds (*Raphanus sativus* var. Long Scarlet) were a commercially available variety (Mr Fothergill's Seeds Pty Ltd).

Seed Treatment. Seeds were surface sterilised in 2% sodium hypochlorite solution for 10 minutes, rinsed 3 times with sterile deionised water and then imbibed for approximately 15 hours in sterile deionised water.

Post-emergence Bioassays. Substances assessed for post-emergence activity were 1,8-cineole **1**, eucalyptus oil, 3-oxo-1,8-cineole **2**; the hydroxylated cineole

compounds **3**, **4a** and **5a**; 1,8-cineole esters **4b-d**; and 1,4-cineole esters **5b** and **c** (Figure 1). The cineole esters assessed for post-emergence activity were selected on the basis of their activity in pre-emergence testing (Barton et al. 2010). Carboxylic acids assessed in these post-emergence bioassays were those corresponding to the esters. Seeds were germinated on sterile water agar in Petri dishes before transfer to the 55 mm Petri dishes that had been prepared with the test compounds. The radish seeds took 24 hours and the ryegrass seeds took 40 hours to germinate when incubated at 25 °C. The water agar was prepared by autoclaving (103.4 kPa, 121 °C, 30 minutes) 4.0 g of agar (BBL^{TM} Agar, Grade A) in 500 mL of deionised water containing calcium (0.05 mol L^{-1}) and boron (0.001 mol L^{-1}). Petri dishes (55 mm plastic) (or Pyrex dishes for chloroform solutions) for the post-emergence bioassays were prepared, under sterile conditions, by pouring the agar into them to a depth of approximately 2 mm and allowing them to solidify. A solution (1 mL) of the test compound in the required solvent (Table 1) was introduced into the Petri dish using a micropipette and the dish left open in a laminar flow cabinet for 3 hours to allow evaporation of the organic solvent. The concentrations for these post-emergence bioassays ranged from concentrations where seedlings showed little or no response to those with complete or nearly complete mortality in pre-emergence bioassays (Barton et al. 2010) (Table 2).

Filter paper bioassays were used for 1,8-cineole and eucalyptus oil. Filter papers (Whatman number 4) were autoclaved, oven dried and placed into autoclaved pyrex Petri dishes (55 mm) under sterile conditions. 1,8-Cineole solution or eucalyptus oil solution (1 mL) was transferred on to the filter paper using a micropipette, the lid placed on the Petri dish and the dish sealed with plastic food wrap. The 1,8-cineole and eucalyptus oil were prepared in aqueous solution with 0.34

mg mL^{-1} of the non-ionic surfactant Tween® 80, polyoxyethylene (20) sorbitan monooleate. The deionised water/Tween® 80 solution, containing calcium and boron as above, was autoclaved prior to preparation of the 1,8-cineole and eucalyptus oil solutions. All glassware used in the preparation of these solutions was washed with 2% sodium hypochlorite solution and then rinsed with sterile deionised water. Ten seedlings were placed in each Petri dish and then sealed with plastic food wrap. The Petri dishes were placed randomly in a tray with Styrofoam supports to angle the dishes at approximately 70° to the horizontal to encourage straighter root and shoot growth. Petri dishes with 1,8-cineole and eucalyptus oil were placed flat. The tray was incubated under light (135 to 195 μE m^{-2} s^{-1} photosynthetic active radiation) at 25 °C for 48 hours. Prior to measurement of the increase in radish root and shoot lengths, seedlings were frozen in their Petri dishes and thawed. This softened their roots and shoots making easier measurement of their lengths. Ryegrass shoots were too fragile to be frozen and thawed.

Two controls, one with and one without solvent, were used for each experiment. For solvent controls, solvent (1 mL) was pipetted on to the surface of the agar and the Petri dish left open in a laminar flow cabinet for three hours. The non-solvent control consisted of the same agar solution in Petri dishes that were similarly left open in a laminar flow cabinet for three hours.

Experimental Design and Data Analysis. Five replicates were used at each concentration and for controls. Petri dishes were placed in a randomised manner in the support tray. Each experiment was repeated in duplicate with two-tailed t-tests showing no significant difference between repeats at $P = 0.05$. Data for effects of concentration on increased root and shoot growth were subjected to one way analysis of variance (ANOVA) using the SPSS 15.0 statistics package (SPSS Inc., 2007).

Differences between means were tested using Tukey's HSD test and were considered to be statistically different at $P < 0.05$. Modelling dose response data using non-linear log-logistic regression analysis to fit it to a sigmoidal curve to determine the I_{50} (50% inhibition) values for root growth and shoot growth was carried out as described by Seefeldt et al. (1995).

Results

These post-emergence bioassays showed that for all tested substances the radish and ryegrass had a dose response with inhibition of root and shoot growth increasing with concentration (Figures 2, 3, 4 and 5). As well, for both plant species the post-emergence bioassays confirmed that reduced root and shoot growths observed in pre-emergence bioassays (Barton et al. 2010) were due to growth inhibition rather than germination delay.

Acids on Radish. Acetic acid suppressed radish root growth and shoot growth at and above 0.01 mol L^{-1} (Figure 2 (a)). Radish roots were more sensitive to benzoic acid than were the shoots with suppression of root growth first observed at 0.000316 mol L^{-1} and for shoot growth at 0.001 mol L^{-1} (Figure 2 (c)). Hexanoic acid inhibited radish root growth at and above 0.0025 mol L^{-1} (Figure 2 (e)). At the highest concentration of 0.05 mol L^{-1}, hexanoic acid reduced root growth by approximately 99%. Radish shoot growth was reduced by hexanoic acid at 0.007 mol L^{-1} reaching 87% reduction compared to the mean of the control at 0.05 mol L^{-1} (Figure 2 (e)).

1,8-Cineole and Eucalyptus Oil on Radish. Suppression of roots and shoots by 1,8-cineole **1** was significant at and above 0.1 mol L^{-1} (Figure 2 (b)). Eucalyptus oil suppressed growth of roots at and above 0.01 g mL^{-1} and shoots above 0.0316 g mL^{-1} (Figure 2 (f)). Roots turned brown and became dehydrated when exposed to 1,8-

cineole **1** or eucalyptus oil at their highest concentrations, and no new root growth occurred.

1,8-Cineole Derivatives on Radish. 3-Oxo-1,8-cineole **2** suppressed shoot growth at and above 0.01 mol L^{-1} whilst root growth was reduced above 0.025 mol L^{-1} (Figure 2 (d)). There was complete inhibition of radish root and shoot growth by 2-*endo*-hydroxy-1,8-cineole **3** at 0.2 mol L^{-1}, the highest concentration tested, as well as significant (for root $P = 1.14 \times 10^{-12}$, for shoot $P = 3.41 \times 10^{-3}$) suppression above 0.05 mol L^{-1} (Figure 3 (a)). At the highest concentration, 2-*endo*-hydroxy-1,8-cineole **3** caused browning at the root tip. 3-*exo*-Hydroxy-1,8-cineole **4a** suppressed ($P = 1.78 \times 10^{-6}$) radish root growth at and above 0.01 mol L^{-1} whilst it only suppressed shoot growth at and above 0.1 mol L^{-1} (Figure 2 (c)). Radish shoots treated with 3-*exo*-hydroxy-1,8-cineole **4a** were clearly lighter green than shoots of the control seedlings. Shoot suppression ($P = 1.87 \times 10^{-4}$) by 3-*exo*-benzoxy-1,8-cineole **4b** was seen at and above 0.01 mol L^{-1} whilst root suppression ($P = 3.05 \times 10^{-3}$) occurred above 0.0316 mol L^{-1} (Figure 2 (e)).

1,4-Cineole Derivatives on Radish. 2-*exo*-Hydroxy-1,4-cineole **5a** suppressed further radish root growth at all the concentrations tested, with growth reducing to 2% of the control mean at 0.1 mol L^{-1} (Figure 3 (b)). This compound only depressed further shoot growth at and above 0.04 mol L^{-1} (Figure 3 (b)). The 2-*exo*-hydroxy-1,4-cineole **5a** caused browning of the radish root tips at 0.1 mol L^{-1}. 2-*exo*-Acetoxy-1,4-cineole **5b** inhibited root and shoot growth only at the highest tested concentration of 0.1 mol L^{-1} (Figure 3 (d)). 2-*exo*-Acetoxy-1,4-cineole, as for 3-*exo*-hydroxy-1,8-cineole, caused shoots to be paler green than shoots of control seedlings. Although 2-*exo*-hexoxy-1,4-cineole **5c** suppressed root and shoot growth at all tested

concentrations it did not completely inhibit further root or shoot growth even at the highest tested concentration (Figure 3 (f)).

Acids on Ryegrass. Acetic acid suppressed post-emergent ryegrass root growth at and above 0.0025 mol L^{-1} with root length decreasing to about 6% of the control mean at 0.05 mol L^{-1} (Figure 4 (a)). Shoot growth was suppressed by acetic acid above 0.01 mol L^{-1} (Figure 4 (a)). *t*-Butylacetylacetic acid suppressed root growth at 0.001 mol L^{-1} ($P = 3.55 \times 10^{-13}$) with complete inhibition of growth at the highest concentration of 0.0316 mol L^{-1} (Figure 4 (c)). Shoots were first suppressed at 0.00316 mol L^{-1} by benzoic acid (Figure 4 (c)). Hexanoic acid suppressed root and shoot growth at and above 0.0025 mol L^{-1} (Figure 4 (e)). This acid completely stopped further root growth at 0.02 mol L^{-1} and shoot growth at 0.05 mol L^{-1} (Figure 4 (e)).

1,8-Cineole and Eucalyptus Oil on Ryegrass. 1,8-Cineole **1** stopped ryegrass root and shoot growth above 0.1 mol L^{-1} with root suppression first occurring at 0.0316 mol L^{-1} ($P = 3.55 \times 10^{-13}$) and shoot suppression at 0.1 mol L^{-1} (Figure 4 (b)). Eucalyptus oil suppressed ryegrass root and shoot growth above 0.00316 g mL^{-1} and completely inhibited root growth above 0.01 g mL^{-1} and shoot growth above 0.0316 g mL^{-1} (Figure 4 (f)).

1,8-Cineole Derivatives on Ryegrass. 3-Oxo-1,8-cineole **2** reduced ryegrass root growth at 0.005 mol L^{-1} whilst shoot growth was decreased above 0.0025 mol L^{-1} (Figure 4 (d)). 2-*endo*-Hydroxy-1,8-cineole **3** reduced root growth above 0.005 mol L^{-1}, with complete inhibition of root growth above 0.1 mol L^{-1} (Figure 5 (a)). 2-*endo*-Hydroxy-1,8-cineole **3** suppressed shoot growth above 0.01 mol L^{-1} (Figure 5 (a)). 3-*exo*-Hydroxy-1,8-cineole **4a** suppressed root growth at and above 0.025 mol L^{-1} leading to complete inhibition above 0.1 mol L^{-1} (Figure 5 (b)). This hydroxy

compound suppressed ryegrass shoot growth above 0.01 mol L^{-1} (Figure 5 (b)). 3-*exo*-Hexoxy-1,8-cineole **4c** completely inhibited ryegrass root growth at and above 0.0316 mol L^{-1} with suppression first observed at 0.01 mol L^{-1} ($P = 6.47 \times 10^{-3}$) but promoted root growth at the two lowest concentrations tested of 0.001 and 0.00316 mol L^{-1} ($P = 2.44 \times 10^{-5}$, and $P = 2.93 \times 10^{-6}$, respectively) (Figure 5 (c)). This compound suppressed ryegrass shoot growth at all concentrations with complete inhibition at the highest concentration (0.1 mol L^{-1}) (Figure 5 (c)). 3-*exo*-*t*-Butylacetoxy-1,8-cineole **4d** reduced root and shoot growth at and above 0.01 mol L^{-1} with roots showing 5% and shoots 27% growth relative to control means at 0.316 mol L^{-1} (Figure 5 (e)).

1,4-Cineole Derivatives on Ryegrass. 2-*exo*-Hydroxy-1,4-cineole **5a** and 2-*exo*-acetoxy-1,4-cineole first suppressed ryegrass root and shoot growth at 0.01 mol L^{-1} with complete root growth inhibition for both at 0.1 mol L^{-1} (Figure 5 (d) and (f)).

The ryegrass roots turned brown and became dehydrated when treated with 1,8-cineole **1**, eucalyptus oil, 2-*endo*-hydroxy-1,8-cineole **3**, 3-*exo*-hexoxy-1,8-cineole **4c**, 2-*exo*-hydroxy-1,4-cineole **5a** and 2-*exo*-acetoxy-1,4-cineole **5b** at their highest tested concentrations. The browning of the roots was apparent within approximately 5 minutes for the 1,8-cineole and eucalyptus oil.

The dose response data for both species closely fitted the sigmoidal curves generated from log-logistic regression analysis (R^2 values all above 0.9) but the I_{50} values are approximate with errors in some cases larger than the estimated I_{50} due to emphasis being on the wide range of compounds tested rather than on repetition to achieve high precision for fewer compounds.

Discussion

As for results of germination bioassays (Barton et al. 2010), the post-emergence results do not support the postulate that cineole esters would be more active than their respective carboxylic acid and the hydroxy cineole due to metabolic cleavage on uptake by plants. In general the post-emergence activity of the cineole esters did not show improvement relative to their respective hydroxylated cineole and carboxylic acid precursors. Eucalyptus oil was compared to the 1,8-cineole **1** effects to give an indication of any effects other components of the oil may have on growth of seedlings. The results suggest limited effect on growth of other components of the oil.

The post-emergent results indicate that for radish, roots were generally more sensitive to the tested substances than were shoots, as also shown by the pre-emergence observations. For the ryegrass, shoots were slightly more sensitive post-emergent but there was no clear trend for sensitivity of roots as compared to shoots for pre-emergence bioassays.

Post-emergence, the carboxylic acids were the most active of the tested substances against radish with 2-*endo*-hydroxy-1,8-cineole **3** as the overall most active of the cineole compounds. Although both 3-*exo*-benzoxy-1,8-cineole **4b** and 2-*exo*-hexoxy-1,4-cineole **5c** initially suppress shoot growth at a lower concentration than 2-*endo*-hydroxy-1,8-cineole **3**, they do not completely inhibit shoot growth even at 1 mol L^{-1} whilst 2-*endo*-hydroxy-1,8-cineole completely inhibits shoots at 0.2 mol L^{-1}. Several cineole compounds initially suppress root growth at a concentration lower than that of 2-*endo*-hydroxy-1,8-cineole but some of these compounds do not give complete root inhibition whilst 2-*endo*-hydroxy-1,8-cineole does.

Of all the cineole compounds 3-*exo*-hexoxy-1,8-cineole **4c** had the highest post-emergence activity against ryegrass with a shoot growth suppression initially

328 occurring at the lowest concentration of the cineole compounds and with complete
329 suppression at 0.1 mol L^{-1}. Whilst other cineole compounds suppressed post-
330 emergent ryegrass root growth at lower concentrations, this hexanoate ester
331 completely inhibited root growth at 0.0316 and 0.1 mol L^{-1}. 2-*endo*-Hydroxy-1,8-
332 cineole **3** was the most active hydroxy-cineole against ryegrass roots but all the
333 hydroxy-cineoles had similar shoot activity.

334 The lighter green of radish shoots treated with 3-*exo*-hydroxy-1,8-cineole
335 compared to that of shoots of control seedlings indicates this compound may interfere
336 with chlorophyll production or enhance its breakdown, or increase production of
337 masking carotenoids. Sing et al. (2002) observed that 1,8-cineole reduced chlorophyll
338 content in billy goat weed as well as reducing cellular respiration. The content of
339 chlorophylls a and b in *Amaranthus viridis* were observed to decrease on treatment
340 with 1,8-cineole, as was the amount of carotenoids (Kaur et al. 2011) suggesting that
341 the lighter green of radish shoots is more likely a result of lowered chlorophyll
342 production rather than presence of masking carotenoids. Kaur et al. (2011) also
343 observed lowered cell respiration in *A. viridis*. The cineole-containing oil of *Ajania*
344 *tenuifolia* caused decreased activity of nitrate reductase and chlorophyll content of
345 *Elymus nutans* (Bai and Zhang 1994). There are many other reports of eucalyptus oils
346 and 1,8-cineole reducing chlorophyll content (Batish et al. 2004; Kohli and Singh
347 1991; Singh et al. 2005). Reduced chlorophyll content will lower photosynthetic
348 efficiency and so contribute to the herbicidal activity of 1,8-cineole. The browning of
349 roots by 1,8-cineole and eucalyptus oil was likely as a result of the volatility of these
350 substances. Reduced root growth may be a result of inhibition of DNA synthesis in
351 nuclei and other organelles in the root apical meristem. 1,8-Cineole has been shown
352 to decrease the DNA synthesis activity in the root tips of *Brassica campestri*

(Koitabashi et al. 1997; Nishida et al. 2005) and to inhibit all stages of mitosis in onion roots (Romagni et al. 2000). The cineole derivatives prepared in this work have higher melting points and lower volatility at ambient temperatures than 1,8-cineole, so overcoming limitations in field use of 1,8-cineole as a herbicide due to its volatility and subsequent low uptake by plants.

Conclusion

In conclusion, there is a dose-dependent post-emergence herbicidal activity by 1,8-cineole and the hydroxy and ester derivatives of 1,8-cineole and 1,4-cineole against radish and annual ryegrass root and shoot growth. 2-*endo*-Hydroxy-1,8-cineole **3** is the most active of the cineole derivatives against radish and 3-*exo*-hexoxy-1,8-cineole **4c** the most active derivative against ryegrass. Many derivatives have improved phytotoxicity relative to 1,8-cineole, particularly at the lower concentrations. Results do not indicate any strong improvement in activity of 1,8-cineole derivatives over 1,4-cineole derivatives. As in the case of pre-emergence bioassays, in this study the carboxylic acids were more active and observed phytotoxicity of ester derivatives may be due to metabolic cleavage of the esters to the hydroxy cineole and carboxylic acid within the plant. Based on these preliminary results, the hydroxyl and ester derivatives of 1,8-cineole and 1,4-cineole have potential as herbicides. However, before further investigation into their potential as herbicides is undertaken it may be most appropriate to assess their mechanism of phototoxicity. A novel mechanism of action may provide stimulus to the development of these potentially safer compounds but research to assess their efficacy in field trials, toxicity against the crop plants that they might be used for and safety would be needed. Structure-activity studies to compare 3-*endo*-hydroxy-1,8-cineole and 3-*exo*-hydroxy-1,8-cineole may also

provide clearer understanding of the position and stereochemical role of hydroxylation of the 1,8-cineole cyclohexane ring.

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529 **Figure captions**

530 **Fig. 1** Structures of the substances used in the post-emergence herbicidal assessments

531

532 **Fig. 2** Effect of (a) acetic acid, (b) 1,8-cineole, (c) benzoic acid, (d) 3-oxo-1,8-
533 cineole, (e) hexanoic acid and (f) eucalyptus oil on post-emergence growth of roots
534 (–◆–) and shoots (–■–) of radish 48 hours after exposure. Bars = means ± SE**;** ***means
535 at and above this concentration were significantly less than (solvent) control means

536

537 **Fig. 3** Effect of (a) 2-*endo*-hydroxy-1,8-cineole, (b) 2-*exo*-hydroxy-1,4-cineole, (c) 3-
538 *exo*-hydroxy-1,8-cineole, (d) 2-*exo*-acetoxy-1,4-cineole, (e) 3-*exo*-benzoxy-1,8-
539 cineole and (f) 2-*exo*-hexoxy-1,4-cineole on post-emergence growth of roots (–◆–)
540 and shoots (–■–) of radish 48 hours after exposure. Bars = means ± SE; ***means at
541 and above this concentration were significantly less than (solvent) control means

542

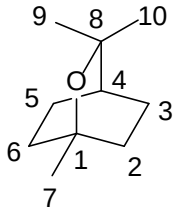
543 **Fig. 4** Effect of (a) acetic acid, (b) 1,8-cineole, (c) *t*-butylacetic acid, (d) 3-oxo-1,8-
544 cineole, (e) hexanoic acid and (f) eucalyptus oil on post-emergence growth of roots
545 (–◆–) and shoots (–■–) of ryegrass 48 hours after exposure. Bars = means ± SE;
546 ***means at and above this concentration were significantly less than (solvent) control
547 means

548

549 **Fig. 5** Effect of (a) 2-*endo*-hydroxy-1,8-cineole, (b) 3-*exo*-hydroxy-1,8-cineole, (c) 3-
550 *exo*-hexoxy-1,8-cineole, (d) 2-*exo*-hydroxy-1,4-cineole, (e) 3-*exo*-*t*-butylacetoxy-1,8-
551 cineole and (f) 2-*exo*-acetoxy-1,4-cineole on post-emergence growth of roots (–◆–)
552 and shoots (–■–) of ryegrass 48 hours after exposure. Bars = means ± SE; ***means at

553 and above this concentration were significantly less than (solvent) control means;

554 means significantly higher than (solvent) control means



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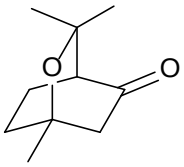

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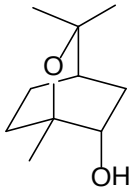

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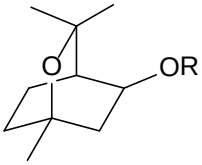
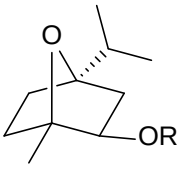


4a R = -H
b R = -COC_6H_5
c R = -$CO(CH_2)_4CH_3$
d R = -$COCH_2(CH_3)_3$



5a R = -H
b R = -$COCH_3$
c R = -$CO(CH_2)_4CH_3$

Figure 1

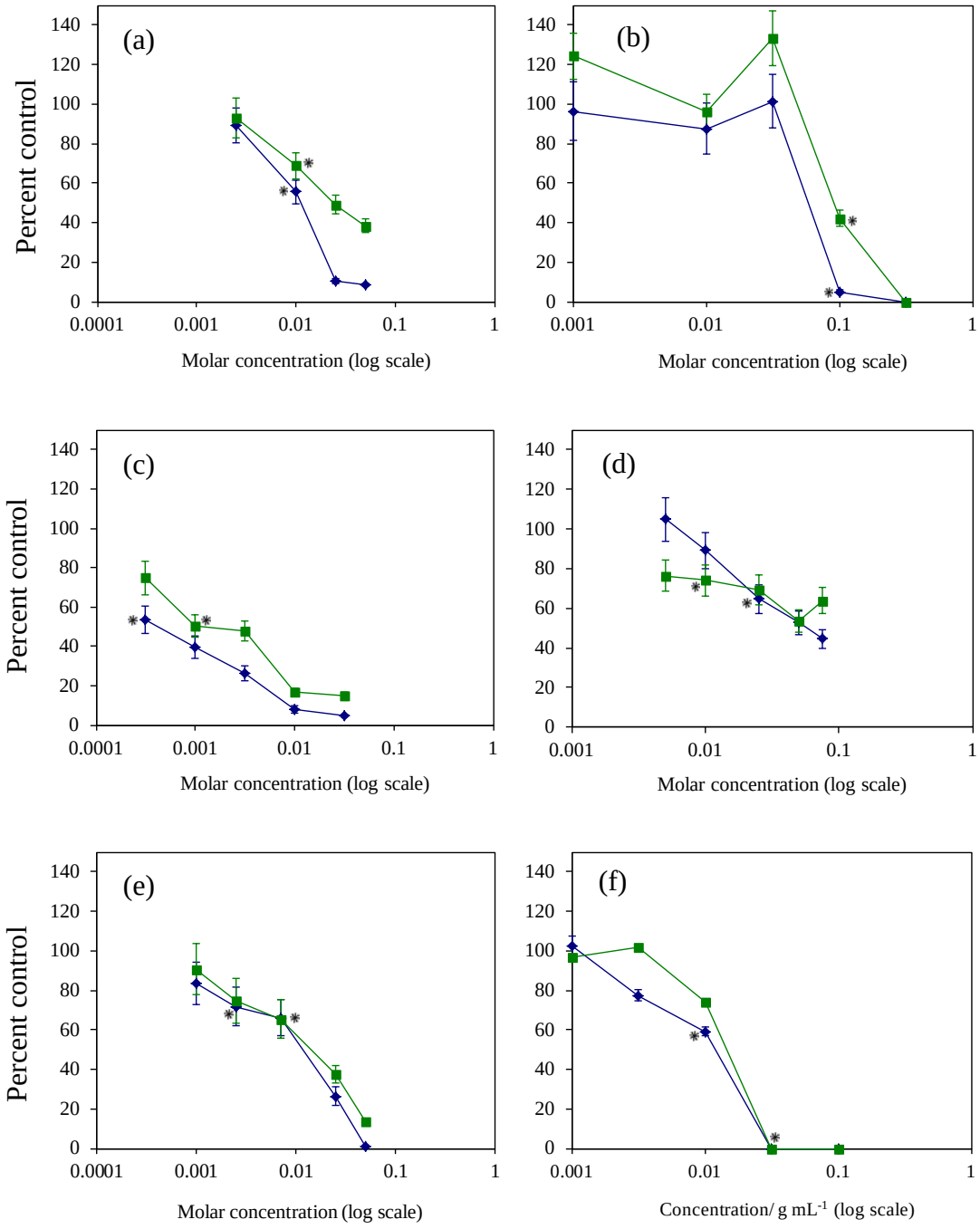

(a)
(b)
(c)
(d)
(e)
(f)
Percent control
Molar concentration (log scale)
Concentration/ g mL-1 (log scale)
0
20
40
60
80
100
120
140
0.0001
0.001
0.01
0.1
1

Figure 2

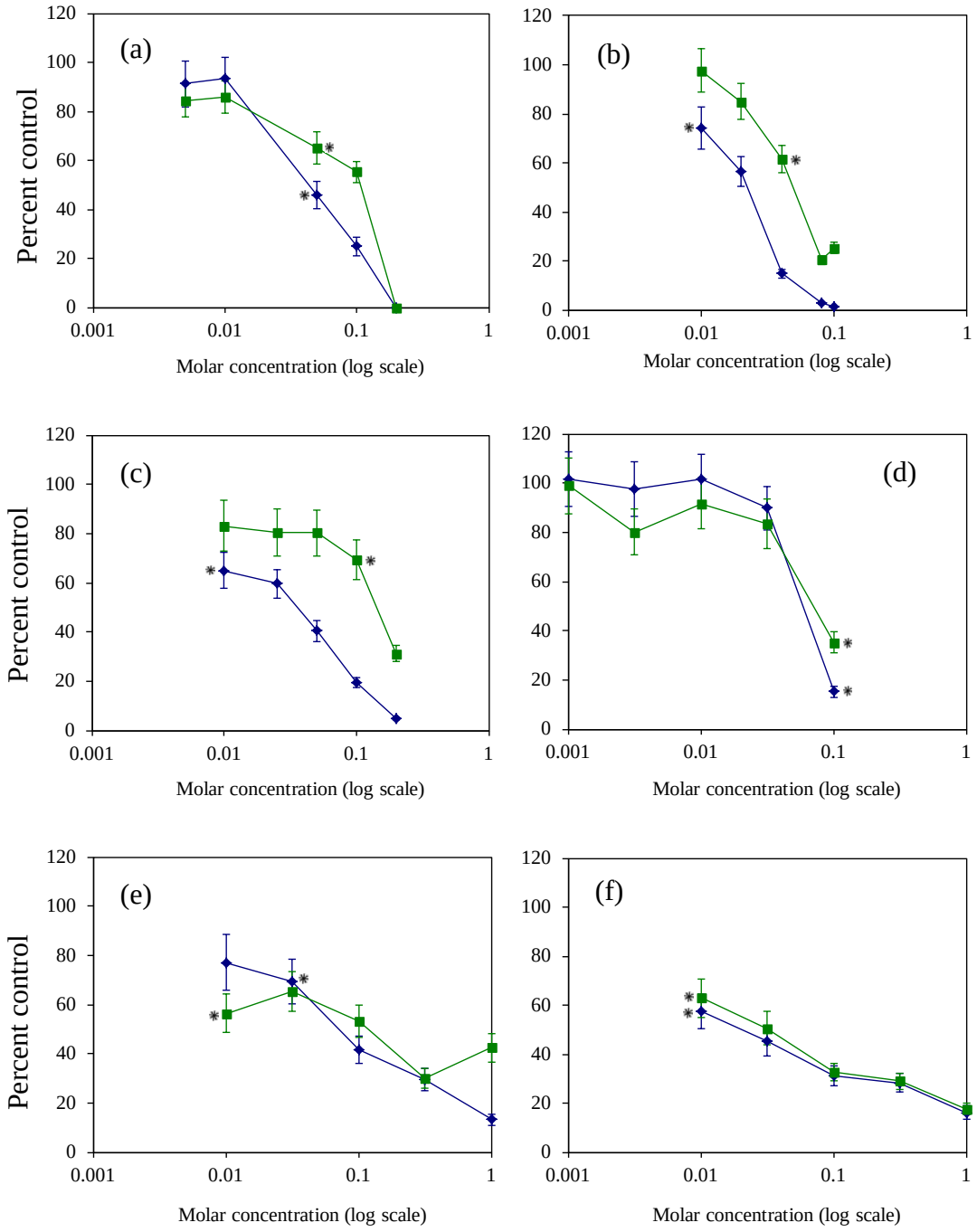

(a)
(b)
(c)
(d)
(e)
(f)
Percent control
Molar concentration (log scale)
120
100
80
60
40
20
0
0.001
0.01
0.1
1

Figure 3

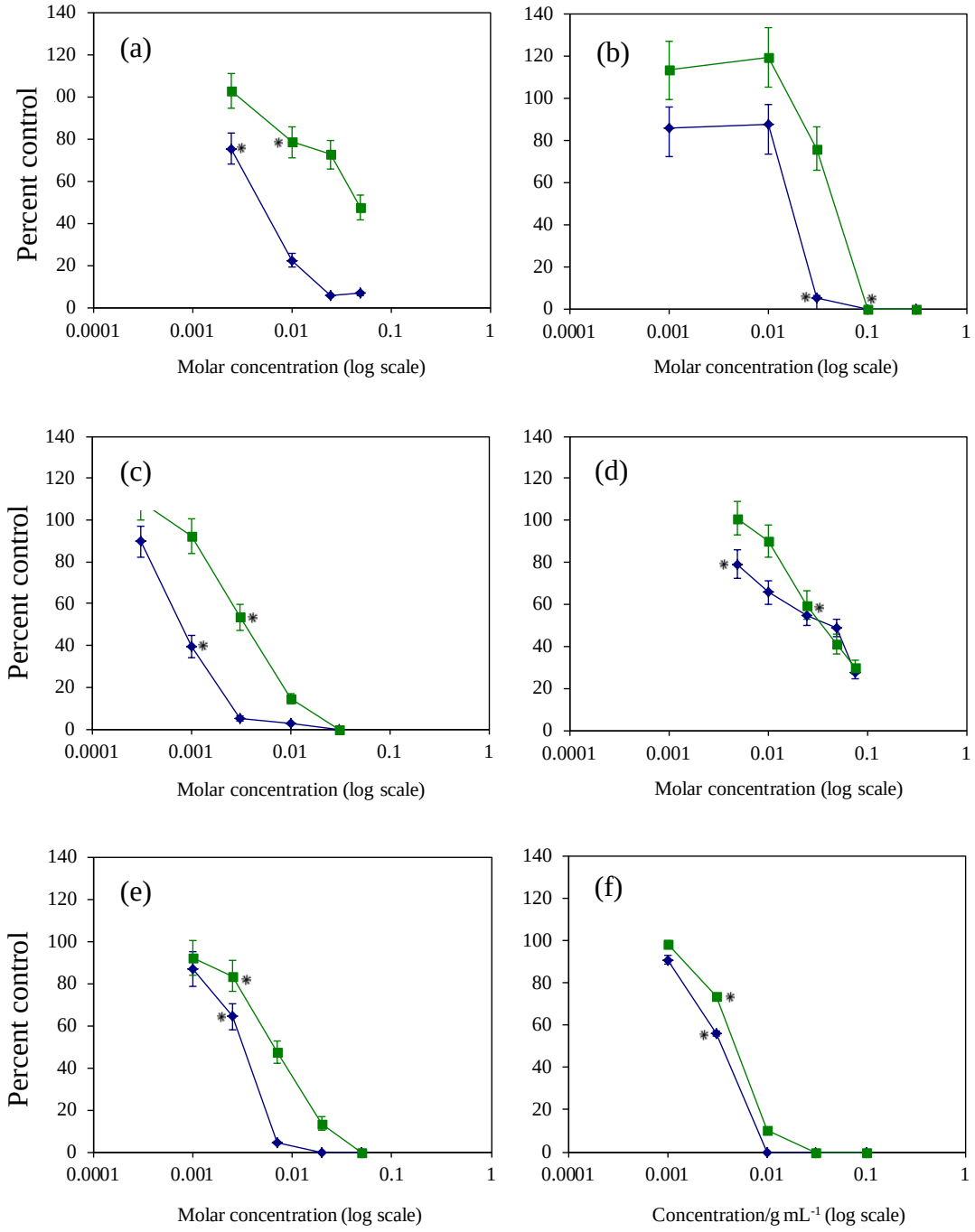

(a)
(b)
(c)
(d)
(e)
(f)
Percent control
Molar concentration (log scale)
Concentration/g mL-1 (log scale)
0
20
40
60
80
100
120
140
0.0001
0.001
0.01
0.1
1

Figure 4

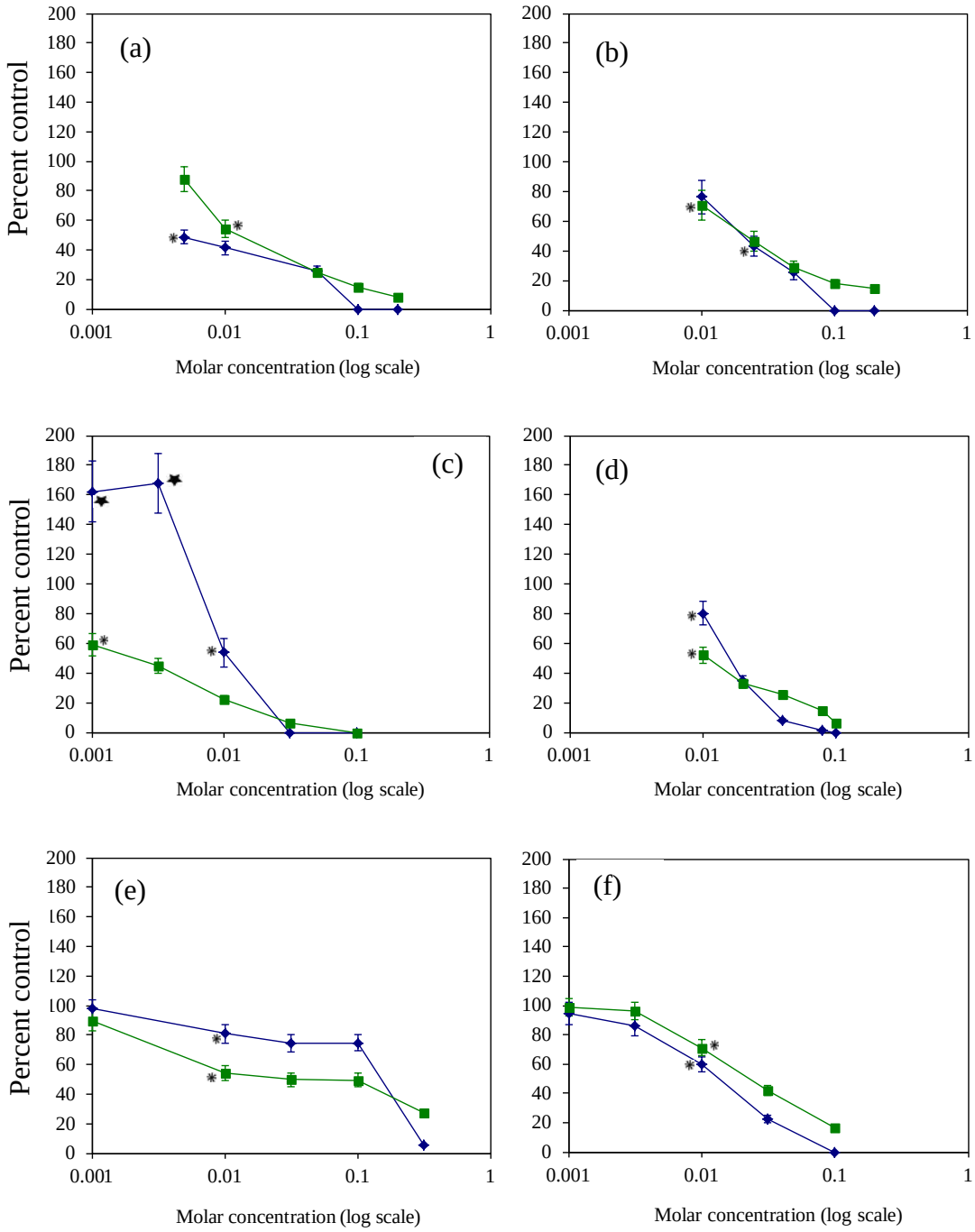

(a)
(b)
(c)
(d)
(e)
(f)
Percent control
Molar concentration (log scale)
0
20
40
60
80
100
120
140
160
180
200
0.001
0.01
0.1
1

Figure 5

Table 1 Solvents used for bioassays of test compounds

Compound	Solvent
acetic acid	water
benzoic acid	trichloromethane (chloroform)
hexanoic acid	hexane
t-butylacetic acid	hexane: chloroform; 99:1
1,8-cineole **1**	Tween® 80 in water (0.34 g L^{-1})
eucalyptus oil	Tween® 80 in water (0.34 g L^{-1})
3-oxo-1,8-cineole **2**	hexane
2-*endo*-hydroxy-1,8-cineole **3**	hexane: chloroform; 99:1
3-*exo*-hydroxy-1,8-cineole **4a**	hexane
3-*exo*-benzoxy-1,8-cineole **4b**	chloroform
3-*exo*-hexoxy-1,8-cineole **4c**	hexane
3-*exo*-*t*-butylacetoxy-1,8-cineole **4d**	chloroform
2-*exo*-hydroxy-1,4-cineole **5a**	hexane: chloroform; 9:1
2-*exo*-acetoxy-1,4-cineole **5b**	hexane
2-*exo*-hexoxy-1,4-cineole **5c**	hexane

Table 2 Concentration of test compounds used in post-emergent herbicidal testing

Compound	Concentrations (mol L^{-1})	
	Radish	**Rye Grass**
acetic acid	0.0025, 0.01, 0.025, 0.05	
benzoic acid	0.000316, 0.001, 0.00316, 0.01, 0.0316	
hexanoic acid	0.001, 0.0025, 0.007, 0.025, 0.05	0.001, 0.0025, 0.007, 0.02, 0.05
t-butylacetic acid		0.000316, 0.001, 0.00316, 0.01, 0.0316
1,8-cineole **1**	0.001, 0.00316, 0.01, 0.0316, 0.1	0.001, 0.01, 0.0316, 0.1, 0.316
eucalyptus oil	[a]0.001, 0.01, 0.0316, 0.1, 0.316	[a]0.001, 0.00316, 0.01, 0.0316, 0.1
3-oxo-1,8-cineole **2**	0.005, 0.01, 0.025, 0.05, 0.075	
2-*endo*-hydroxy-1,8-cineole **3**	0.005, 0.01, 0.05, 0.1, 0.2	
3-*exo*-hydroxy-1,8-cineole **4a**	0.01, 0.025, 0.05, 0.1, 0.2	
3-*exo*-benzoxy-1,8-cineole **4b**	0.01, 0.0316, 0.1, 0.316, 1.0	
3-*exo*-hexoxy-1,8-cineole **4c**		0.001, 0.00316, 0.01, 0.0316, 0.1
3-*exo*-*t*-butylacetoxy-1,8-cineole **4d**		0.001, 0.01, 0.0316, 0.1, 0.316
2-*exo*-hydroxy-1,4-cineole **5a**	0.01, 0.02, 0.04, 0.08, 0.1	
2-*exo*-acetoxy-1,4-cineole **5b**	0.001, 0.00316, 0.01, 0.0316, 0.1	
2-*exo*-hexoxy-1,4-cineole **5c**	0.01, 0.0316, 0.1, 0.316, 1	

[a] Concentration units for eucalyptus oil solution is g mL^{-1}